

# Seed addition and biomass removal key to restoring native forbs in degraded temperate grassland

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## Abstract

### Questions

Long-term restoration of native forb diversity can only be achieved if native forb species can recruit (colonise and establish) and reproduce. We asked whether native forbs in a temperate grassland were seed limited, and how the recruitment of native and exotic forbs is affected by grassland structure and resource availability.

### Location

Australian Capital Territory, south-eastern Australia.

## Methods

We conducted a field experiment in a temperate grassland dominated by a native tussock grass to assess effects of: 1) addition of native forb seed, 2) thinning of native grass tussocks, 3) leaf litter removal, and 4) exotic plant removal on the recruitment of native and exotic forbs. These four actions can alter grassland structure and the availability of soil nutrients, soil moisture, and light. We used generalised linear mixed models to determine the importance of seed addition, grassland structure and resource availability on the richness and abundance of sown native forbs, and the abundance of exotic forb seedlings and unsown native forbs.

## Results

Adding seed increased the species richness and abundance of native forbs. Tussock thinning and litter removal increased species richness and abundance of sown native forbs, and the abundance of exotic forb seedlings. Exotic plant removal also increased the abundance of sown native forbs. Abundance of unsown native forb species was unaffected by the experimental treatments. Species richness and abundance of native forbs and abundance of exotic forbs declined with increasing tussock grass cover. Leaf litter restricted the abundance of native forb species more than exotic forb species.

## Conclusion

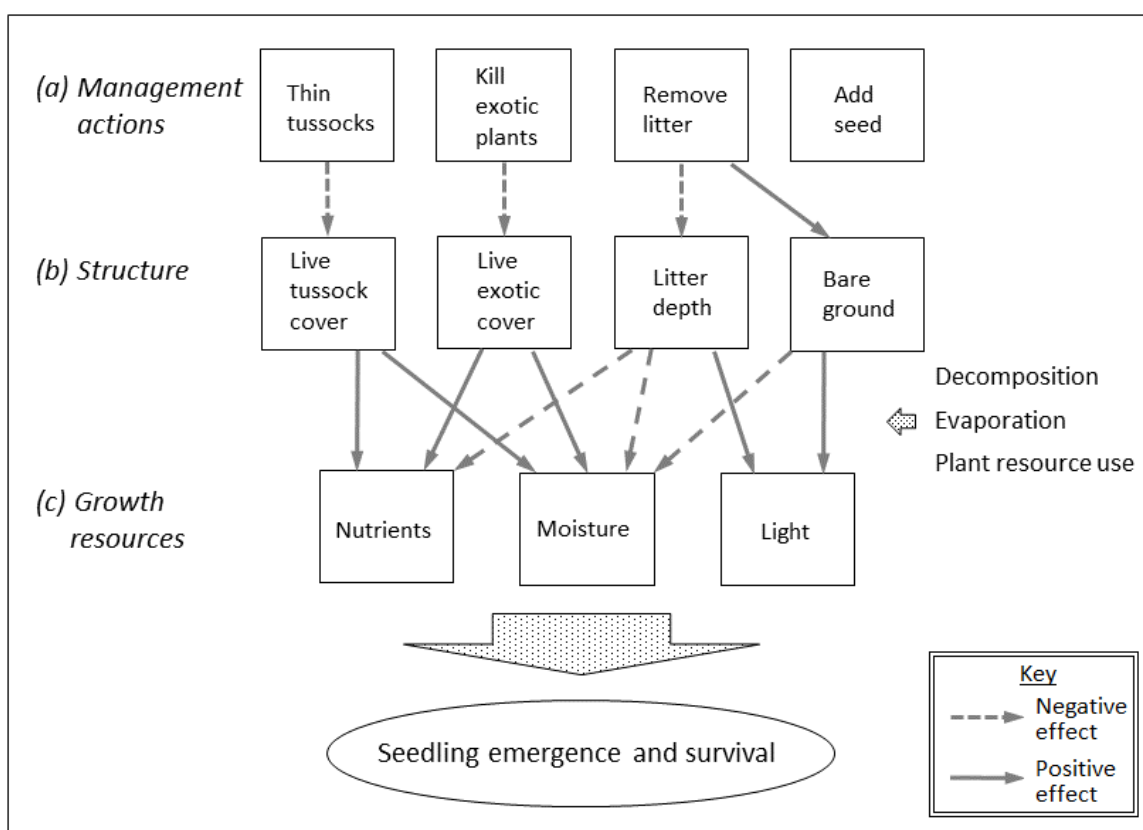
Native forb recruitment predominantly relied upon seed addition, suggesting that seed limitation is a major barrier to the recovery of degraded grasslands. Reducing the cover of living grass tussocks facilitated recruitment of native and exotic forbs, and removing litter disproportionately increased recruitment of native forbs compared with exotics. Combining seed addition with the reduction of both living and dead grass biomass should help restore native grassland forbs.

## Introduction

Forb diversity has declined in many parts of the world following the conversion of grasslands for agriculture, and often remains low even when agriculture is subsequently abandoned (Wheeler et al. 2015; Fensham et al. 2016). Forbs represent a large proportion of plant species richness in natural grasslands (Tremont & McIntyre 1994; Klimek et al. 2007; Jacquemyn et al. 2011; Mitchell & Bakker 2016) and their decline reduces the functional diversity of grassland ecosystems (Hooper et al. 2005). The decline of grassland diversity has flow-on impacts on pollinator diversity (Wilkerson et al. 2014), resistance to invasion (Hulvey & Zavaleta 2012), and the availability of habitat resources for other grassland dependent taxa (Barrett et al. 2008). Restoring and maintaining forb diversity is thus a key conservation goal for grasslands around the world (Hobbs et al. 2013). The Australian Government lists the ecosystem under consideration in this study as Critically Endangered, largely on the basis of lost forb diversity (<https://www.environment.gov.au/system/files/resources/be2ff840-7e59-48b0-9eb5-4ad003d01481/files/box-gum.pdf>; accessed 12-10-2017).

Low rates of seedling emergence and survival may explain why few forb species recover once agriculture has ceased (Fayolle et al. 2009; Donath & Eckstein 2010). Poor seedling emergence and survival can result from limited availability of both seeds and other resources that are needed for early survival and growth (Zobel et al. 2000; Brandt & Seabloom 2012; Dybzinski & Tilman 2012). Seed supply may be limited because of an absence of source populations, poor dispersal from source populations, and the lack of a persistent soil seedbank (Brandt & Seabloom 2012). If forb species are seed-limited, increasing seed availability (e.g. through direct seeding) will be required (Jacquemyn et al. 2011; Morgan & Williams 2015). Even where adequate seed is present, recruitment requires resources essential for plant growth (light, nutrients and moisture), which may be influenced by grassland structure and competition (Morgan 1998b; Hellström et al. 2009; Frances et al. 2010).

Understanding the ways in which different management actions affect native and exotic forb recruitment will help optimise restoration (Fig. 1). For example, the actions needed to restore native forb diversity will differ if they are physically restricted by accumulated leaf litter, or if they are resource-limited due to competition from established vegetation (Moles & Westoby 2004). If restricted by litter or competition from native grasses, litter removal and control burns would be effective management actions (Fynn et al. 2005), whereas weed control would be more effective in the case of restriction by exotic species (Prober & Thiele 2005). Understanding how exotic forbs respond to management is also important because management actions designed to increase native forb recruitment may also inadvertently facilitate exotic species recruitment (Faithfull et al. 2012; Driscoll 2017).



**Fig. 1.** Hypothesised impacts of management actions on grassland structure, resource availability, and native forb recruitment. Management actions (a) change grassland structure (b), and structural changes influence resource availability (c) by initiating changes in the rates of decomposition, evaporation and plant resource use. The changes flowing from each of these management actions are hypothesised to have a positive effect on seedlings.

Temperate grasslands in south-eastern Australia exemplify the challenges faced when attempting to restore forb diversity in grasslands. Agriculture practices over the last 200 years have drastically simplified Australian grasslands, with fertiliser application, herbicide use, sowing of exotic pasture grasses, livestock grazing, and suppression of fires all being common practice. As a result, less than 0.5% of the original extent of diverse grassland ecosystems remains (Prober et al. 2013), and remnant grassland is now isolated in small fragments (Tremont & McIntyre 1994), limiting opportunities for seed dispersal among patches.

In this experimental study, we implemented four treatments (thin tussocks, kill exotic species, remove litter, and add seed) that represent key management actions for restoring grassland forbs. Our aims were to: i) assess the necessity of seed addition for the re-establishment of native forbs, ii) determine which treatments, structural components and resources are associated with

native forb recruitment (Fig. 1), and iii) compare how the abundance of native forbs and exotic forbs respond to these treatments.

## Methods

### Study site

We conducted the experiment in a temperate grassland in the Australian Capital Territory (ACT) in south-eastern Australia (35.270562° S, 149.026425° E, 574 m above sea level). The site was located in a nature reserve, and has a median annual rainfall of 650 mm. The 1.5 km<sup>2</sup> reserve was previously a pastoral lease starting with sheep grazing from the 1920s and more recently (1985-2005) low intensity cattle grazing. It was declared a nature reserve in 2010. In 2011 and 2012, a non-residual selective broadleaf herbicide (fluroxypyr) was applied (via boom-spraying) in parts of the reserve to control (successfully in the short-term) an invasive exotic forb, *Hypericum perforatum*.

We established the experiment on a gentle (5 degrees), south-facing slope that was dominated by *Themeda triandra* (Kangaroo grass) before the experiment began. *Themeda triandra* is a summer growing native perennial tussock grass, which can become dominant when present. In productive conditions, *T. triandra* accumulates a thick mat of dead leaf litter—from leaves that die over winter—if not removed (e.g. by fire). The litter was up to 3 cm deep in parts of the study site, with an average dry mass of 310 g.m<sup>-2</sup>. Before the start of the experiment, 43 native forb species (up to 10 species per 0.5 m<sup>2</sup>) were observed across the whole reserve (Johnson 2013, Unpubl.), but only 10 forb species (up to 4 species per 0.5 m<sup>2</sup>) were observed in the experimental site, probably due to previous herbicide use and lack of disturbance. Exotic grasses and forbs are abundant in the surrounding area (>2 m from experimental site), but made only a minor contribution to vegetation in the experimental site.

### Experimental design

The experiment was a fully crossed factorial design with all 16 (2<sup>4</sup>) treatment combinations randomly arranged within each of six blocks, making a total of 96 plots (Table 4 in Appendix S1). The plots, measuring 0.75 m x 0.75 m and separated by at least 75 cm, were located within a single 1000 m<sup>2</sup> area of homogenous grassland to minimise variation due to topography, soils, weather, and vegetation type. The area was fenced to minimise grazing by vertebrate herbivores (kangaroos, rabbits).

The experimental plots within each block were randomly treated with every combination of: (a) *T. triandra* tussocks thinned by ~50% by spraying with glyphosate (7.2 g.L), (b) all leaf litter on the

ground removed by hand, (c) all exotic grasses and forbs killed by painting individuals with glyphosate (7.2 g.L), and (d) addition of seed for 14 native forb species that occur naturally in the region (Tables 3 and 4 in Appendix S1). Three of the sown species already occurred in the site. Plants killed with glyphosate were not removed. All treatments were applied in the austral spring (5-6 Oct 2014), and exotic plant removal was done three more times: late-spring (Nov 2014), summer (Jan 2015) and early autumn (Mar 2015). The plots were watered with a known amount applied evenly using a spray nozzle and a timer as required to encourage germination in the first month, and after that at a rate equivalent to the 75<sup>th</sup> percentile of historic rainfall to simulate a good growing season.

#### Response variables

The four response variables were: i) the species richness of sown native forbs (age < 8 months); ii) the abundance (count of individuals) of sown native forbs; iii) the abundance (count) of unsown exotic forbs; and iv) the abundance (count) of unsown native forbs (Table 5a in Appendix S1). Response variables were measured in the central 0.5 m x 0.5 m of each 0.75 m x 0.75 m plot to avoid edge effects. For sown native forbs, we used the maximum counts from two surveys conducted in early summer and early winter, 11 weeks and eight months after the treatments, respectively, as the best indication of total recruitment over that period. Count averages were not possible because of difficulty determining the season in which individuals had emerged. We did not measure the species richness of exotic forbs as individuals were too small to identify to species-level (Table 8c in Appendix S2).

#### Explanatory variables

We measured six potential explanatory variables to characterise physical structure and resource availability within each plot.

Physical structure within the central 0.5 m x 0.5 m of each plot was represented by i) the percentage area covered by living grass tussocks, not including attached tussock biomass that had senesced due to the thinning treatment or natural die-back over winter; ii) ground litter depth averaged from three measurements; and iii) the visually estimated percentage area of bare ground (Table 5b in Appendix S1). These data were recorded separately for each quarter of a 0.5 m x 0.5 m quadrat, and the results subsequently averaged. We did not include the cover of exotic species as a structural explanatory variable because exotic species made up little cover (mean 2.2%  $\pm$  SD 7.1%) prior to the final months of the experiment.

Resource availability was represented by: i) the percentage of light penetrating the canopy to above the litter layer, measured with a LI-COR LI-191 line quantum sensor positioned along both

diagonals of the 0.75 m x 0.75 m square plots; ii) available soil phosphorus measured within a  $\text{NaHCO}_3$  extract of the soil using a Lachat QuikChem 8500 flow injection analyser; and iii) the percentage of soil moisture, by volume, measured in the outer 12 cm on opposing sides of each plot with a Delta-T Theta Probe ML3 moisture probe four days after rain (Table 5b in Appendix S1). Soil sampling and moisture measurement was done in the outer 12 cm to avoid disturbance to the central  $0.25 \text{ m}^2$  where the plant responses and structural variables were measured.

We also measured the oven-dry weight ( $80^\circ \text{C}$ ) of litter lying on the ground—consisting almost entirely of dead tussock leaves—gathered from twelve untreated  $0.25 \text{ m}^2$  patches, two beside each block, to estimate the overall litter biomass.

### Statistical analysis

We used generalised linear mixed models (GLMM) within the “glmmTMB” package in R statistical software (R Core Team 2016) to model relationships between plant responses to: (a) the experimental treatments, and (b) to explanatory covariates representing physical structure and the availability of resources within each plot that are potentially affected by the treatments. Responses to treatments and covariates were modelled separately as there were strong correlations between the tussock thinning treatment and the percent cover of living tussock ( $r = -0.65$ ), and between the litter removal treatment and litter depth ( $r = -0.86$ ). All combinations and subsets of the structural variables (percent cover of living tussock, litter depth, and bare ground) and resource variables (light penetrating the canopy, available soil phosphorus, and soil moisture) were evaluated as potential model terms. Total tussock cover was excluded due to correlation with cover of living tussock ( $r = 0.82$ ). Light at ground level was excluded due to correlation with light penetrating the canopy ( $r = 0.72$ ) and bare ground ( $r = 0.71$ ). The remaining potential explanatory variables were not highly correlated ( $r < 0.7$ ).

We used a Poisson distribution with log-link function to model sown native forb species richness, and negative binomial distributions for plant abundance data because they were more widely dispersed than Poisson distributions due to high numbers of zeros and several high scores. Of the 96 plots in the experiment, 48 were sown with native forbs. Only data from the 48 sown plots were used to model sown native forb responses, because no sown native forbs were found in the unsown plots. We modelled exotic forb responses using data from the 48 plots where the exotic removal treatment was applied to ensure that counts were of seedlings only. Data from all 96 plots were used to model the response of unsown native forb species.

We fitted block number, representing the individual blocks in which treatments were grouped, as a random effect term to account for variation between blocks. We used Bayesian

Information Criterion (BIC) information criteria to determine the fixed terms of the ‘best fit’ models, out of all possible non-correlated combinations. We calculated *Delta BIC*—the difference in BIC between the ‘best fit’ and the ‘best fit minus one term’ models—as a basis for comparing the relative influence of individual terms (Burnham & Anderson 2003).

## Results

We recorded a total of 4465 forb individuals: 1264 seedlings for nine of the 14 sown native forb species (Table 8a in Appendix S2), 2001 individuals of exotic species (including 900 seedlings in plots where exotic species had been removed), and 1200 unsown native forb individuals of any age (Table 8b in Appendix S2).

Prior to establishing the treatments, native species richness (mean  $1.1 \pm \text{SD } 1.1$ ) and percent cover of native species ( $6.2\% \pm 10.7\%$ ) across all plots (not including *T. triandra*) was higher than for exotic species ( $0.3 \pm 0.6$ , and  $2.2\% \pm 7.1\%$ ).

### Responses to treatments

There were statistically significant associations between three of the four response variables (sown native forb richness, sown native forb abundance and exotic forb abundance) and one or more of the treatments (Table 1, Fig. 2). Seedlings of the sown native forb species only emerged where seed had been added, and with no other treatments mean total native richness and abundance increased by 122% and 61% respectively. Where seed was added, mean total richness and abundance of native forbs increased with tussock thinning (+214%, +78%), litter removal (+160%, +64%), or both (+575%, +488%) (Table 4 in Appendix S2).

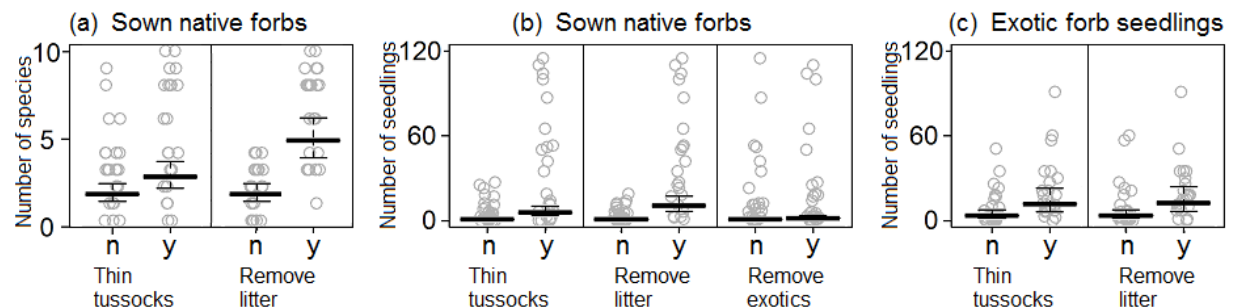
Sown native species richness and abundance, and exotic abundance were positively associated with tussock thinning and litter removal. Sown native abundance was also positively associated with exotic species removal (Table 1a-c, Fig. 2a-c). Litter removal had a greater influence than tussock thinning on the richness (*Delta BIC* = 49 vs 8) and abundance (*Delta BIC* = 41 vs 25) of sown native forbs (Table 1a-b). However, litter removal and tussock thinning had similar influence on the abundance of exotic forbs (*Delta BIC* = 9 vs 8) (Table 1c). There were no additional effects from interactions between treatments.

**Table 1.** GLMM models fitted to predict the effects of the experimental treatments on: (a) sown native forb species richness (seeded plots only), (b) sown native forb abundance (seeded plots only) and (c) exotic forb seedling abundance (exotic removal plots only). There were no significant models



predicting the response of (d) unsown native forb abundance to the treatments (all plots). Block number was included as a random effect in each model.

Plant group response	BIC	df	Treatment	Estimate	Std. Err.	F pr.	Delta BIC
(a) Sown native forb species richness	204.2	43	Thin tussocks	0.498	0.146	<0.001	8
			Remove litter	1.105	0.163	<0.001	49
(b) Sown native forb abundance	353.6	42	Thin tussocks	1.697	0.201	<0.001	25
			Remove litter	2.284	0.240	<0.001	41
			Remove exotics	0.492	0.149	<0.001	6
(c) Exotic forb abundance	367.9	43	Thin tussocks	1.152	0.311	<0.001	8
			Remove litter	1.212	0.317	<0.001	9
(d) Unsown native forb abundance	N/A						



**Fig. 2.** Effects of the three treatments (thin tussocks, remove litter, remove exotics) on (a) sown native forb species richness, (b) sown native forb abundance and (c) exotic forb abundance.

Predicted responses (mean  $\pm$  95% confidence intervals) to individual treatments (y), assuming all other treatments are controls (n), were obtained from GLMMs in which the block number was fitted as a random effect. Observed values are indicated by open circles [two observed counts not shown – maximum (b)(193) and maximum (c)(188)].

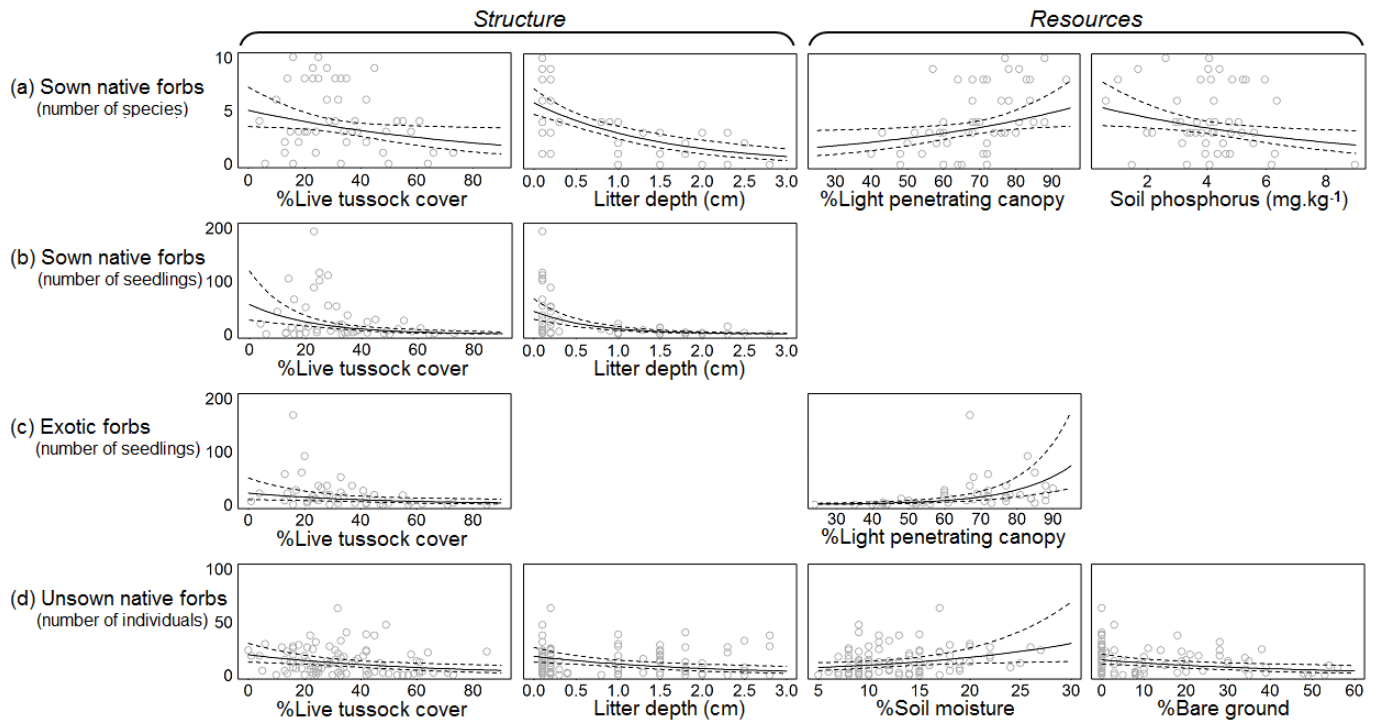
### Responses to structure

Sown native species richness and abundance, and unsown native forb abundance were significantly negatively associated with the explanatory variables representing grassland structure (live tussock cover and litter depth). Litter depth had the greatest relative influence on these models (*Delta BIC* = 26, 27 and 5 respectively). In addition, unsown native forb abundance was negatively associated with the percent area of bare ground. Exotic forb abundance was associated with live tussock cover but not litter depth (Table 2a-d, Fig. 3a-d). Weak correlations between live tussock cover and the availability of the measured resources (light penetrating the canopy,  $r = -0.38$ ; light at ground level,  $r = 0.00$ ; phosphorus,  $r = -0.13$ ; soil moisture,  $r = -0.24$ ) suggest the effects of live tussock cover cannot be explained by its effect on these resources. A negative correlation between litter depth and light

at ground level ( $r = -0.85$ ) demonstrates a strong shading effect, but weak correlations between litter and the other measured resources (phosphorus,  $r = 0.00$ ; and soil moisture,  $r = 0.33$ ) suggested the effect of litter was not because of its influence on these resources.

**Table 2.** GLMM models predicting the responses of: (a) sown native forb species richness (seeded plots only), (b) sown native forb abundance (seeded plots only), (c) exotic forb seedling abundance (exotic removal plots only), and (d) unsown native forb abundance (all plots) to the experimental covariates. Block number was included as a random effect in each model.

Plant group response	Covariate	BIC	df	Estimate	Std. Err.	F Pr.	Delta BIC
(a) Sown native forb species richness	%Live tussock cover	206.7	42	-0.012	0.005	0.031	1
	Litter depth			-0.671	0.134	<0.001	26
	%Light penetrating canopy			0.017	0.007	0.021	1
	Phosphorus			-0.124	0.051	0.014	2
(b) Sown native forb abundance	%Live tussock cover	371.5	43	-0.044	0.011	<0.001	9
	Litter depth			-1.475	0.204	<0.001	27
(c) Exotic forb abundance	%Live tussock cover	352.7	43	-0.022	0.010	0.027	1
	%Light penetrating canopy			0.065	0.012	<0.001	17
(d) Unsown native forb abundance	%Live tussock cover	686.3	89	-0.016	0.006	0.005	3
	Litter depth			-0.530	0.173	0.002	5
	Soil moisture			0.058	0.025	0.019	0
	Bare ground			-0.022	0.008	0.007	3



**Fig. 3.** Predicted responses to structural and resource variables (mean  $\pm$  95% confidence intervals) from the fitted GLMMs for a) sown native forb species richness, b) sown native forb abundance, c) exotic forb seedling abundance and d) unsown native forb abundance. Predictions were calculated across the range of values observed for each fixed effect in the GLMM with other fixed effects held at their mean. Observed values are indicated by open circles.

#### Responses to resource availability

Sown native forb species richness was positively associated with the percent light penetrating the canopy and negatively associated with soil phosphorus. Sown native forb abundance was not associated with any of the measured resources. Unsown native forb abundance was positively associated with soil moisture. Exotic forb seedling abundance was positively associated with percent light penetrating the canopy, which had a greater influence on this response than percent live tussock cover ( $\Delta BIC = 17$  vs 1), the structural variable in that model. In all other models, resource availability had a relatively minor influence compared with structure (Table 2a-d, Fig. 3a-d).

## Discussion

We investigated whether the addition of seed is needed to restore native forbs in a temperate grassland; and compared responses by native and exotic forbs to tussock thinning, litter removal and weed control. We found that the richness and abundance of native forbs were significantly positively associated with the addition of seed, removal of living biomass (native tussock grasses and exotic species) and removal of litter. Our results agreed with studies conducted in other parts of the world that demonstrate the negative influence that competition from established plants and litter can have on forb seedling recruitment (Dybinski & Tilman 2012; Scott & Morgan 2012; Loydi et al. 2013). We found that native forb seedlings were more restricted by litter than exotic forb seedlings, and thus, controlling litter build-up is essential for maintaining the richness and abundance of native forbs. However, our results indicated that tussock thinning and litter removal will not result in successful establishment of native forb species without an adequate supply of seed.

#### The need for seed

Seed addition will probably be necessary when restoring forb diversity in degraded temperate grasslands, as there is little potential for restoring lost forb diversity through natural dispersal (Hobbs & Yates 2003; Heinken & Weber 2013). While the use of herbicides may have reduced the supply of naturally occurring native forb seed at the experiment site, small and fragmented populations of

persisting species are universal symptoms of grassland degradation (Hobbs & Yates 2003). And lost species above ground are also likely to be lost from the soil seedbank (Morgan 2001). Clark et al. (2007) argue that the availability of suitable sites is more limiting than seed limitation for recruitment in grasslands, but both were important in our experiment. The richness and abundance of native forbs increased with seed addition and the amount of increase depended on which other treatments were applied (Table 4 in Appendix S2).

Recruitment of the three sown species that were already present at the site relied entirely on seed addition, and nine of the 11 unsown native forb species recorded in our plots (Table 8c in Appendix S2) also failed to recruit. This is probably due to seed limitation as tussock thinning and litter removal had created many sites suitable for germination and establishment (Clark et al. 2007; Dybzinski & Tilman 2012). We cannot be sure of the age of the extant forbs or when the last natural recruitment event occurred, but the unsown native forbs on our site (predominantly perennial) may not have successfully recruited for many years—perhaps since the last major disturbance event (Lauenroth & Adler 2008) 12 years earlier.

Seed limitation is common in plant communities and often occurs in combination with a limitation of resources needed for germination and establishment (Eriksson & Ehrlén 1992; Clark et al. 2007). The arrival of seed from source populations may be affected by interactions between landscape factors (e.g. habitat and population fragmentation and isolation, pollinator availability) and species attributes (e.g. population sizes, dependence on pollinators, genetic self-compatibility/incompatibility) (Hobbs & Yates 2003; Aguilar et al. 2006; Heinken & Weber 2013). Dispersal success is influenced by seed characteristics (e.g. size, dispersal appendages), release height (Thomson et al. 2011), and landscape and site conditions (Soons et al. 2005). Dispersal over time is limited by seedbank longevity, which for Australian native forb species is generally short (Morgan 1998a). Our results suggest that seed for the sown native species and almost all the unsown existing native species were neither present in the seedbank nor dispersing to the site in sufficient quantities—probably a consequence of insufficient numbers of reproductive individuals within dispersal range (Nathan & Muller-Landau 2000; Scott & Morgan 2012).

### Structural influence

Structure influences the recruitment of native forbs directly through physical effects and indirectly by moderating the availability of resources (Davis et al. 2000). Tussocks and litter take up space and create a physical barrier that can restrict seedling emergence (Donath & Eckstein 2010) or prevent seeds from reaching mineral soil (Ruprecht & Szabó 2012). Live tussock cover influences forb recruitment indirectly by competing for available soil resources and light (Dybzinski & Tilman 2012;

Loydi et al. 2015). Litter reduces seedling emergence indirectly by reducing the amount of light at ground level (Foster & Gross 1998), although accumulated leaf litter can also be beneficial for seedling emergence in dry conditions through temperature moderation and increased water retention (Loydi et al. 2013).

Exotic forb seedling abundance increased with litter removal, but we found that the abundance of sown native forb seedlings benefited relatively more than exotic species from litter removal than other treatments (based on *Delta-BIC*, Table 1b-c). We were unable to determine the degree to which litter depth restriction on native seedlings was related to physical obstruction or the availability of light, but exotic seedlings were not significantly restricted by litter depth. Our results suggest that exotic forb seedlings can cope with a greater litter depth. Therefore, in productive grasslands where litter accumulates, periodic removal of litter build-up is essential for maintaining the richness and abundance of native forb species as a persistent litter layer will favour the recruitment of exotic species over natives, leading to an increased proportion of exotic forbs. There were no positive forb seedling responses to litter in our study; even the abundance of established unsown native forbs was negatively associated with the litter depth existing before the experiment.

#### Response to resource availability

Although the richness and abundance of native forbs generally exhibited the strongest associations with structural attributes of grassland (i.e., litter depth and tussock cover), there were significant associations with some of the measured resources. For example, native forb species richness was negatively associated with soil phosphorus and positively associated with light penetrating the canopy (measured above the litter). Negative associations between elevated soil phosphorus (e.g., from the application of fertilizer or introduction of livestock) and the richness of native forbs has been widely observed (Dorrough & Scroggie 2008; Seabloom et al. 2015; Morgan et al. 2016). Most native species are unable to compete with exotic species in soils with high soil phosphorus levels, as many exotic species evolved in, and are better adapted to, soils high in phosphorus (Daehler 2003). Increased light penetrating the canopy benefits seedling and adult forbs that have grown above the litter, and it may also benefit seedlings that need light to grow through the litter (by increasing the amount of light penetrating into the litter), in this way reducing the severity of litter restriction, as per Hautier et al. (2009). The abundance of exotic forbs was more strongly associated with the amount of light penetrating the canopy than the depth of the litter. Exotic forb species in our study are generally better adapted for rapid growth and therefore have a greater need for resources, including light (Borer et al. 2014; Neuenkamp et al. 2016).

While native forb seedling abundance was not associated with resource availability, most seedlings were counted while very small, and it is likely that resources would become more limiting with increasing competition among growing seedlings.

#### Implications for management

Experimental seed addition increased the richness and abundance of native forbs, especially when combined with tussock thinning and litter removal. Living grass tussock cover can be reduced by fire, selective herbicide application, or physically removing a proportion of individual plants. Litter build-up can also be minimised by periodic burning, or physical removal. Grazing can also reduce grass cover and litter build-up, but frequent grazing may be counter-productive as it leads to a reduction in native forbs and an increase in exotic species (Dorrough et al. 2004). Care should be taken that management actions to reduce grass cover and litter build-up do not exceed thresholds required by vulnerable grassland biota. For example, Howland et al (2014) found that the species richness and abundance of ground-dwelling reptiles declined following a change in grassland structure caused by grazing. However, environmental thresholds are likely to be species-specific and may require additional research and choices of which species to favour.

The removal of exotic species was also found to benefit sown native forb abundance to some extent, even though exotic species were initially scarce at our site (Table 1b, Fig. 2b). The removal of exotic species is likely to cost less and be more effective in the long-term if populations are removed while small (Rejmánek & Pitcairn 2001; Simberloff et al. 2013). It would also be preferable to control exotic species before taking actions to reduce tussock cover or litter depth, as reduced biomass may encourage the expansion of existing exotic species.

A key result of our study was that litter restricted the abundance of emerging sown native forb seedlings more than exotic forb seedlings. The most likely reason for this is that Australian grassland species have evolved where the amount of litter was generally less than in Europe—due to greater biomass productivity in European grass species (Groves et al. 2003), and a lower likelihood of fire that removes litter (Bond et al. 2005). Indeed, we demonstrated negative impacts on native forbs where the average ground litter mass was greater than 310 g.m<sup>-2</sup>, considerably less than the 500 g.m<sup>-2</sup> threshold suggested by Loydi et al (2013) based on research carried out mainly in Europe and USA. *Themeda triandra* grasslands, found mainly in the southern hemisphere (Hodgkinson et al. 1989), were poorly represented in the meta-analysis by Loydi et al (2013). It is reasonable to conclude that litter levels can directly influence the composition of native and exotic forbs in grassland communities, and grasslands with litter levels above the native tolerance threshold are likely to become progressively dominated by exotic forbs. Such a trend may trigger a positive

feedback mechanism accelerating the decline of native forbs, due to increased exotic competition for resources and a proportionally reduced native seed supply. Strategic use of litter removal on sites dominated by native tussock grasses combined with the addition of native forbs in spring can benefit native forb richness and abundance in preference to exotic forb abundance; initially by reducing litter restriction on emerging native forbs, and subsequently through greater seed supply and competition for resources from an increased presence of native species.

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#### Supporting Information

Appendix S1. Tables related to experiment design and results.

Appendix S2. List of species.

**Appendix S1.** Tables related to experiment design and results.

Table 3. Experimental treatments and their descriptions. Treatments were not applied in the control plots for each treatment.

<i>Treatment</i>	<i>Description</i>
(a) Thin tussocks	50% of tussock plants were randomly sprayed with glyphosate (concentration 7.2 g.L), using a bottomless plastic plant pot as a spray shield. The sprayed biomass was left standing.
(b) Remove litter	All leaf litter on the ground was removed by hand.
(c) Remove exotic plants	All exotic plants were daubed with glyphosate (concentration 7.2 g.L) and left to die in situ so as not to disturb the soil. This was done four times during the 8 month experiment.
(d) Add seed	Seed for 14 native forb species from the region (Appendix 2) were mixed together and scattered by hand while sheltered from the wind. The vegetation and litter was then agitated by hand to encourage seeds to fall through to the ground. A high seeding rate (0.7g per plot = 1.2 g.m <sup>-2</sup> , per species) was used to minimise failure from seed limitation. The 14 species were selected because they are readily available for restoration projects in south eastern Australia.

Table 4. Sixteen treatment/control combinations with average total native forb species richness and abundance. Percentage increase in Seeded plots is shown in brackets.

Average total native forb:			Species richness (% increase)	Abundance (% increase)	
Tussocks NOT thinned	Litter NOT removed	Exotics NOT removed	No Seed	1.5	11.0
			Seed	3.3 (122%)	18 (61%)
		Exotics removed	No Seed	1.0	3.5
			Seed	2.8 (183%)	15 (338%)
	Litter removed	Exotics NOT removed	No Seed	1.7	12.3
			Seed	4.3 (160%)	20 (64%)
		Exotics removed	No Seed	1.8	13.3
			Seed	7.0 (282%)	34 (154%)
Tussocks thinned	Litter NOT removed	Exotics NOT removed	No Seed	1.2	14.3
			Seed	3.7 (214%)	26 (78%)
		Exotics removed	No Seed	2.5	12.0
			Seed	4.3 (73%)	20 (67%)
	Litter removed	Exotics NOT removed	No Seed	1.3	12.7
			Seed	9.0 (575%)	75 (488%)
		Exotics removed	No Seed	2.7	11.7
			Seed	10.3 (288%)	113 (867%)
		All Plots	No Seed	1.7	11.4
			Seed	5.6 (228%)	40 (252%)

Table 5. Variables used in the analysis. Response variables (a), and explanatory variables (b).

(a) Response variables		
	Definition	Collection method
<i>SRSown</i>	Species richness of sown native forb species.	Maximum species richness from the summer and autumn surveys.
<i>noSown</i>	Number of sown native forbs.	Maximum seedling count from the summer and winter surveys. The maximum was used because we were interested in emergence, not survival.
<i>noExotic</i>	Number of exotic forb seedlings.	Counted in the autumn survey. Very few exotic seedlings had emerged at the time of the spring survey.
<i>noOthNtv</i>	Number of unsown native forb plants.	Counted in the autumn survey.
(b) Explanatory variables		
	Definition	Collection method
<i>Litter depth</i>	Ground litter depth (cm).	Average depth of litter on the ground for the area not covered by tussock.
<i>%Live tussock</i>	Percentage of area covered by living tussocks.	Average of the estimated percentage of live (green) tussock cover in each plot quarter, estimated mid-summer.
<i>%Light penetrating canopy</i>	Percentage of the total photosynthetically active radiation (PAR) penetrating the tussock canopy.	Measured with a one metre long LI-COR LI-191 line quantum sensor. Total PAR was measured above the canopy, and penetrating PAR was the average of two readings above the litter layer, one for each plot diagonal.
<i>Phosphorus</i>	Available soil phosphorus (mg/kg).	Two soil samples were taken from opposite sides of the plot in the outer 10cm, then bulked together. Available phosphorus was measured within a NaHCO <sub>3</sub> extract of the soil using a Lachat QuikChem 8500 flow injection analyser.
<i>%Soil moisture</i>	Percentage of soil moisture by volume.	The average of two readings by a Delta-T Theta Probe ML3 taken on opposite sides of the plot in the outer 10cm. Measured once, all on the same day, four days after rain, as an indicator of soil moisture holding capacity within each plot.
<i>%Bare ground</i>	Percent of area that is bare ground.	Average of the estimated percentage area of bare ground in each quarter.

Table 6. Overall summary statistics, for (a) all response variables, and (b) significant explanatory variables.

(a) Response variable	Min	Max	Mean (SD)	(b) Explanatory variable	Min	Max	Mean (SD)
<i>SRSown</i>	0	10	4.2 (3.0)	<i>Litter depth (cm)</i>	0.1	2.8	0.9 (0.9)
<i>noSown</i>	0	193	26.3 (40.4)	<i>%Live tussock cover</i>	0	85	33 (17)
<i>noExotic</i>	0	188	22.9 (34.3)	<i>%Light penetrating canopy</i>	24	94	66 (15)
<i>noOthNtv</i>	0	63	12.5 (12.3)	<i>Phosphorus (mg.kg)</i>	0.6	8.9	4.1 (1.4)
				<i>%Soil moisture</i>	5	27	13 (5)
				<i>%Bare ground</i>	0	56	11 (16)

Table 7. Mean (and standard deviation) for (a) response variables and (b) significant explanatory variables in the full data set, by treatment.

(a) Response variables	Thin-Ctrl	Tussocks thinned	Litter-Ctrl	Remove litter	Exotics-Ctrl	Remove exotics	Seed-Ctrl	Add seed
<i>SRSown</i>	1.6 (2.3)	2.6 (3.5)	1.0 (1.5)	3.1 (3.6)	1.9 (2.6)	2.3 (3.2)	0 (0)	4.2 (3.0)
<i>noSown</i>	3.8 (7.0)	22.5 (41.5)	2.4 (4.4)	24.0 (41.2)	10.2 (23.1)	16.1 (37.4)	0 (0)	26.3 (40.0)
<i>noExotic</i>	11.7 (17.6)	30.0 (38.7)	13.4 (20.1)	28.3 (38.2)	22.9 (33.9)	18.8 (28.6)	20.0 (33.3)	21.7 (29.4)
<i>noOthNtv</i>	12.1 (14.4)	12.9 (9.6)	12.5 (11.6)	12.5 (12.8)	13.3 (12.7)	11.7 (11.7)	11.4 (10.8)	13.6 (13.5)
<u>(b) Explanatory variables</u>								
<i>Phosphorus (mg.kg)</i>	4.1 (1.3)	4.1 (1.4)	4.2 (1.4)	4.0 (1.3)	4.2 (1.5)	4.0 (1.2)	4.1 (1.3)	4.1 (1.5)
<i>%Live tussock cover</i>	44.1 (16.0)	21.7 (9.6)	30.5 (18.2)	35.3 (16.0)	32.0 (16.7)	33.8 (17.8)	33.5 (18.1)	32.3 (16.3)
<i>Litter depth (cm)</i>	0.9 (0.9)	0.9 (0.8)	1.6 (0.6)	0.1 (0.1)	1.0 (0.9)	0.8 (0.8)	1.0 (0.9)	0.8 (0.8)
<i>%Bare ground</i>	7.6 (11.4)	14.9 (17.9)	0.5 (1.5)	22.1 (15.6)	9.4 (13.2)	13.2 (17.2)	10.6 (14.8)	11.9 (16.1)
<i>%Soil moisture</i>	12.6 (4.7)	13.3 (4.9)	14.6 (5.3)	11.2 (3.4)	12.0 (4.4)	13.8 (4.9)	13.5 (4.9)	12.4 (4.6)
<i>%Light penetrating canopy</i>	58.8 (13.3)	73.7 (11.7)	60.8 (14.4)	71.7 (12.6)	67.6 (12.7)	64.9 (16.1)	64.0 (16.2)	68.6 (12.3)

**Appendix S2.** List of species.

Table 8. (a) Sown native species. Name, family, life form, life cycle, seeds per gram, and maximum germination (\* = already present in low numbers, \*\* = not present at the site but present in the nature reserve, \*\*\* = not previously found in the reserve). (b) Unsown native species. Name, family, life form, life cycle. (c) Unsown exotic species. Name life cycle, and species origin.

Species		Family	Life form	Life cycle	Est. seeds.g	Max germ
<u>(a) Sown native forbs</u>						
<i>Bulbine bulbosa</i>	*	Asphodelaceae	Geophyte	Perennial	528	2.4%
<i>Calocephalus citreus</i>	***	Asteraceae	Chamaephyte	Perennial	10,387	-
<i>Chrysocephalum apiculatum</i>	**	Asteraceae	Hemicryptophyte-Partial	Perennial	6,750	0.3%
<i>Chrysocephalum semipapposum</i>	**	Asteraceae	Hemicryptophyte-Proto	Perennial	4,889	0.5%
<i>Craspedia variabilis</i>	**	Asteraceae	Hemicryptophyte-Partial	Perennial	2,833	0.9%
<i>Cullen tenax</i>	***	Fabaceae	Chamaephyte	Perennial	156	-
<i>Dichopogon fimbriatus</i>	**	Anthericaceae	Geophyte	Perennial	465	-
<i>Eryngium ovium</i>	*	Apiaceae	Hemicryptophyte-Proto	Perennial	306	-
<i>Glycine tabacina</i>	**	Fabaceae	Hemicryptophyte-Proto	Perennial	156	-
<i>Leucochrysum albicans</i>	***	Asteraceae	Hemicryptophyte-Erect	Perennial	1,316	1.4%
<i>Linum marginale</i>	***	Linaceae	Hemicryptophyte-Proto	Perennial	826	3.8%
<i>Plantago varia</i>	*	Plantaginaceae	Hemicryptophyte-Erect	Perennial	461	5.9%
<i>Vittadinea muelleri</i>	**	Asteraceae	Hemicryptophyte-Proto	Perennial	943	10.0%
<i>Wahlenbergia stricta</i>	**	Campanulaceae	Hemicryptophyte-Proto	Perennial	18,500	0.2%
<u>(b) Unsown native species previously existing at the site</u>						
<i>Bulbine bulbosa</i>		Asphodelaceae	Geophyte	Perennial		
<i>Cheilanthes austrotenuifolia</i>		Pteridaceae	Hemicryptophyte-Proto	Perennial		
<i>Cymbonotus preissianus</i>		Asteraceae	Hemicryptophyte-Flat	Perennial		
<i>Eryngium ovium</i>		Apiaceae	Hemicryptophyte-Proto	Perennial		
<i>Eucalyptus spp</i>		Myrtaceae	Phanerophyte	Perennial		
<i>Geranium solanderi</i>		Geraniaceae	Hemicryptophyte-Proto	Perennial		
<i>Hydrocotyle laxiflora</i>		Apiaceae	Hemicryptophyte-Erect	Perennial		
<i>Leptorhynchus squamatus</i>		Asteraceae	Hemicryptophyte-Partial	Perennial		
<i>Lomandra filiformis</i>		Lomandraceae	Hemicryptophyte-Erect	Perennial		
<i>Plantago varia</i>		Plantaginaceae	Hemicryptophyte-Erect	Perennial		
<i>Triptilodiscus pygmaeus</i>		Asteraceae	Therophyte	Annual		
<u>(c) Unsown exotic species</u>						
	<u>Life cycle</u>	<u>Origin (<a href="http://plantnet.rgbsyd.nsw.gov.au">http://plantnet.rgbsyd.nsw.gov.au</a>, accessed 10/1/2016)</u>				
<i>Conyza bonariensis</i>	Annual	South America				
<i>Centaurium tenuiflorum</i>	Annual	Europe				
<i>Cirsium vulgare</i>	Biennial	North Africa, Europe, Asia				
<i>Galium divaricatum</i>	Annual	Europe to south east Asia				
<i>Hypochaeris glabra</i>	Annual	Europe, Asia, Africa				
<i>Hypochaeris radicata</i>	Perennial	Europe				
<i>Hypericum perforatum</i>	Perennial	Asia, Europe, Africa				
<i>Lactuca serriola</i>	Biennial	Europe, Asia				
<i>Linaria pelisseriana</i>	Annual	South Europe				
<i>Myosotis discolor</i>	Annual / Biennial	Europe				

<i>Perforrhagia nanteuillii</i>	Annual	Europe
<i>Plantago lanceolata</i>	Annual / Biennial	Europe, north and central Asia
<i>Tolpis barbata</i>	Annual	Mediterranean
<i>Tragopogon dubius</i>	Biennial	Europe
<i>Trifolium spp</i>	Annual	Europe, Mediterranean, Middle East, north Africa, west Asia